that double-stranded RNA corresponding to an insect gene target sequence produced in either wild-type or RNaseIII-deficient bacterial expression systems is toxic towards the insect in terms of substantial increases in insect mortality and growth/development delay for larval survivors. It is also clear from these experiments that an exemplification is provided for the effective protection of plants/crops from insect damage by the use of a spray of a formulation consisting of bacteria expressing double-stranded RNA corresponding to an insect gene target.

Example 10

Chilo suppressalis (Rice Striped Stem Borer)

A. Cloning of Partial Sequence of the *Chilo suppressalis* Genes Via Family PCR

[0409] High quality, intact RNA was isolated from the 4 different larval stages of *Chilo suppressalis* (rice striped stem borer) using TRIzol Reagent (Cat. Nr. 15596-026/15596-018, Invitrogen, Rockville, Md., USA) following the manufacturer's instructions. Genomic DNA present in the RNA preparation was removed by DNase treatment following the manufacturer's instructions (Cat. Nr. 1700, Promega). cDNA was generated using a commercially available kit (SuperScript™ III Reverse Transcriptase, Cat. Nr. 18080044, Invitrogen, Rockville, Md., USA) following the manufacturer's instructions.

[0410] To isolate cDNA sequences comprising a portion of the CS001, CS002, CS003, CS006, CS007, CS009, CS011, CS013, CS014, CS015, CS016 and CS018 genes, a series of PCR reactions with degenerate primers were performed using Amplitaq Gold (Cat. Nr. N8080240, Applied Biosystems) following the manufacturer's instructions.

[0411] The sequences of the degenerate primers used for amplification of each of the genes are given in Table 2-CS. These primers were used in respective PCR reactions with the following conditions: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 55° C. and 1 minute at 72° C., followed by 10 minutes at 72° C. The resulting PCR fragments were analyzed on agarose gel, purified (QIAquick Gel Extraction kit, Cat. Nr. 28706, Qiagen), cloned into the pCR4/TOPO vector (Cat. Nr. K2500-20, Invitrogen), and sequenced. The sequences of the resulting PCR products are represented by the respective SEQ ID NO:s as given in Table 2-CS and are referred to as the partial sequences. The corresponding partial amino acid sequences are represented by the respective SEQ ID NO:s as given in Table 3-CS.

B. dsRNA Production of the *Chilo suppressalis* Genes

[0412] dsRNA was synthesized in milligram amounts using the commercially available kit T7 Ribomax™ Express RNAi System (Cat. Nr. P1700, Promega). First two separate single 5' T7 RNA polymerase promoter templates were generated in two separate PCR reactions, each reaction containing the target sequence in a different orientation relative to the T7 promoter.

[0413] For each of the target genes, the sense T7 template was generated using specific T7 forward and specific reverse primers. The sequences of the respective primers for amplifying the sense template for each of the target genes are given in Table 8-CS. The conditions in the PCR reactions were as follows: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 55° C. and 1 minute at 72° C., followed by 10 minutes at 72° C. The anti-sense T7 template was generated using specific forward and specific T7 reverse primers in a PCR reaction with the same conditions as described above. The sequences of the respective primers for amplifying the anti-sense template for each of the target genes are given in Table 8-CS. The resulting PCR products were analyzed on agarose gel and purified by PCR purification kit (Qiaquick PCR Purification Kit, Cat. Nr. 28106, Qiagen) and $NaClO_4$ precipitation. The generated T7 forward and reverse templates were mixed to be transcribed and the resulting RNA strands were annealed, DNase and RNase treated, and purified by sodium acetate, following the manufacturer's instructions. The sense strand of the resulting dsRNA for each of the target genes is given in Table 8-CS.

C. Laboratory Trials to Test dsRNA Targets, Using Artificial Diet for Activity Against *Chilo suppressalis* Larvae

[0414] Rice striped stem borers, *Chilo suppressalis*, (origin: Syngenta, Stein, Switzerland) were maintained on a modified artificial diet based on that described by Kamano and Sato, 1985 (in: Handbook of Insect Rearing. Volumes I & II. P Singh and RF Moore, eds., Elsevier Science Publishers, Amsterdam and New York, 1985, pp 448). Briefly, a litre diet was made up as follows: 20 g of agar added to 980 ml of Milli-Q water and autoclaved; the agar solution was cooled down to approximately 55° C. and the remaining ingredients were added and mixed thoroughly: 40 g corn flour (Polenta), 20 g cellulose, 30 g sucrose, 30 g casein, 20 g wheat germ (toasted), 8 g Wesson salt mixture, 12 g Vanderzant vitamin mix, 1.8 g sorbic acid, 1.6 g nipagin (methylparaben), 0.3 g aureomycin, 0.4 g cholesterol and 0.6 g L-cysteine. The diet was cooled down to approx. 45° C. and poured into rearing trays or cups. The diet was left to set in a horizontal laminair flow cabin. Rice leaf sections with oviposited eggs were removed from a cage housing adult moths and pinned to the solid diet in the rearing cup or tray. Eggs were left to hatch and neonate larvae were available for bioassays and the maintenance of the insect cultures. During the trials and rearings, the conditions were 28±2° C. and 80±5% relative humidity, with a 16:8 hour light:dark photoperiod.

[0415] The same artificial diet is used for the bioassays but in this case the diet is poured equally in 24 multiwell plates, with each well containing 1 ml diet. Once the diet is set, the test formulations are applied to the diet's surface (2 cm^2), at the rate of 50 μl of 1 μg/μl dsRNA of target. The dsRNA solutions are left to dry and two first instar moth larvae are placed in each well. After 7 days, the larvae are transferred to fresh treated diet in multiwell plates. At day 14 (i.e. 14 days post bioassay start) the number of live and dead insects is recorded and examined for abnormalities. Twenty-four larvae in total are tested per treatment.

[0416] An alternative bioassay is performed in which treated rice leaves are fed to neonate larvae of the rice striped stem borer. Small leaf sections of Indica rice variety Taichung native 1 are dipped in 0.05% Triton X-100 solution containing 1 μg/μl of target dsRNA, left to dry and each section placed in a well of a 24 multiwell plate containing gellified 2% agar. Two neonates are transferred from the rearing tray to each dsRNA treated leaf section (24 larvae per treatment). After 4 and 8 days, the larvae are transferred to fresh treated rice leaf sections. The number of live and dead larvae are assessed on days 4, 8 and 12; any abnormalities are also recorded.